Published in final edited form as:
Curr Opin Chem Biol. 2008 October ; 12(5): 491–496. doi:10.1016/j.cbpa.2008.06.035.

Microelectrodes for Studying Neurobiology

Justin M. Kita[1] and **R. Mark Wightman**[1,*]

1*Department of Chemistry and Neuroscience Center, University of North Carolina, Chapel Hill, NC 27599-3290, USA*

Abstract

Summary of recent advances—Microelectrodes have emerged as an important tool used by scientists to study biological changes in the brain and in single cells. This review briefly summarizes the ways in which microelectrodes as chemical sensors have furthered the field of neurobiology by reporting on changes that occur on the sub-second time scale. Microelectrodes have been used in a variety of fields including their use by electrophysiologists to characterize neuronal action potentials and development of neural prosthetics. Here we restrict our review to microelectrodes that have been used as chemical sensors. They have played a major role in many important neurobiological findings.

Introduction

Microelectrodes have been used to elucidate a number of neurobiological questions. For example, microelectrodes have been used to probe the basics of vesicular release at the cellular level using a technique known as constant potential amperometry [1]. More recently, however, fast-scan cyclic voltammetry (FSCV) has emerged as one of the primary alternatives to microdialysis for studying neurobiology due to its less invasive implantation and enhanced temporal resolution [2]. Both techniques have proven critical for furthering our understanding of brain chemistry and its role in behavioral neurobiology.

Sensors for measuring neurobiological events must be both small and rapid. Microelectrodes are thus ideal for this type of application since they possess dimensions in the micron range [3]. Microelectrodes can be fabricated in a variety of ways, either through insulation of a carbon fiber with a glass capillary, or utilizing carbon fiber nanoelectrodes [4]. The small size of the electrode minimizes the double-layer capacitance, thus making it possible to make recordings on the sub-second time scale [5]. Microelectrodes are not inherently selective; however, the selectivity of the microelectrode can be enhanced either through application of techniques such as FSCV or chemical modification of the electrode surface.

Electrochemical detection utilizing microelectrodes requires that the detected species be electroactive. Several neurochemical transmitters can be electrochemically detected, such as dopamine and serotonin and many other molecules derived from the amino acids tyrosine and tryptophan. Additionally, there are other processes, such as transient changes in physiological pH, which can be detected electrochemically using FSCV. Microelectrodes are also capable of measuring changes in concentration of several biologically relevant gases such as nitric oxide [6], oxygen [7], and carbon monoxide [8]. In the case of FSCV, multiple analytes can

Correspondence: R. Mark Wightman, Department of Chemistry, Venable Hall CB 3290, University of North Carolina, Chapel Hill, NC, 27599-3290, USA, Tel: +1 (919) 962-1472, Fax: +1 (919) 962-2388, E-mail: rmw@unc.edu.

be detected simultaneously, and when combined with sophisticated data analysis procedures, the individual concentrations can be accurately measured as a function of time [9].

Many biological changes, particularly at the protein level, occur on the order of minutes; however, many biological changes also occur on the order of milliseconds. Early neurobiological techniques such as microdialysis were limited to measuring tonic changes, or changes that occur over several minutes. This limitation prevented further study of neurobiological mechanisms, which can alter chemical communication within a matter of a few milliseconds. These changes can be monitored with microelectrodes either through amperometry or voltammetry [10].

Microelectrodes are now widely used in numerous biological preparations, ranging from single cell to awake, behaving animals. This versatility permits neuroscientists to probe a variety of neurobiological mechanisms such as exocytosis, neuron receptor functionality, and the chemical changes that occur in addiction to drugs of abuse. This review will provide specific, recent examples of how microelectrodes can be used to measure rapid biological and chemical changes.

Investigating exocytosis using amperometry

Neurons communicate with each other chemically through a process known as exocytosis, where one cell is electrically stimulated to elicit the release of neurotransmitters which can then diffuse to neighboring cells to initiate a biological action. Microelectrodes have significantly advanced our understanding of the exocytosis process [11]. Measuring the released neurotransmitters requires a detection method that is both rapid and sensitive enough to measure zeptomole quantities. By holding a microelectrode at a constant potential that is sufficient to cause electrooxidation of any nearby molecules, the current can be measured and the number of electroactive molecules contacting the microelectrode can be quantitated [12]. This process is termed constant potential amperometry, and can be used at the cellular level to determine a variety of neurobiological parameters, such as vesicular neurotransmitter content, frequency and kinetics of neurotransmitter release, and exocytotic release mechanisms [13, 14]. Because of the dimensions of the microelectrode, it can be placed sufficiently close to the cell to ensure complete oxidation of the extruded molecules. This allows the exact amount of neurotransmitter contained within each vesicle to be determined [15], as well as to measure the frequency of firing under different conditions [16]. Amperometry has been used to determine the role of various ion channels in the exocytotic process. By measuring the frequency and kinetics of individual release events, the role of the Ca^{2+} channel was found to have separate effects on fusion pore binding and dilation [17].

Information on the organization of the internal structure of the neuron is typically provided by other common neurobiological techniques such as electron and fluorescence spectroscopy. In particular these techniques have been used to determine the compartmentalization of neuronal vesicles into three distinct subpopulations: readily releasable, recycling, and reserve pools [18,19]. Microelectrodes have been used to confirm these results by individually accessing each of the compartments and measuring the resulting neurotransmitter release from the reserve pool [20]. Further amperometric studies have investigated the proteins implicated in the exocytotic machinery. Genetic silencing of one of these proteins, synaptotagmin I, led to a decreased amount of fractional release of both dopamine and norepinephrine [21]. Further work with the protein synapsin demonstrated that deletion of synapsin ultimately led to an increased number of catecholamine release events, and that overexpression of synapsin led to a decreased number of release events [20]. These amperometric measurements suggest that synapsin acts as an endogenous negative regulator of catecholamine release. Amperometry provides a reliable means of verifying the results obtained through other preparations.

As these examples show, amperometry is a valuable technique that is useful for studying fundamental systems such as cellular preparations. The sub-second temporal resolution of amperometry makes it an ideal technique for measuring the kinetics of exocytotic events, and the small size of microelectrodes makes measuring the quantal size of neurotransmitter vesicles possible. Through direct measurement of released neurotransmitters, amperometry provides a complementary means of studying the effects of cellular proteins on exocytosis.

Investigating full neuronal circuitry using voltammetry

Where microelectrodes truly excel is when they are coupled with FSCV to detect electroactive neurotransmitters directly. This technique takes full advantage of the microelectrode's inherent superior spatial and temporal resolution in addition to the selectivity provided by cyclic voltammetry. FSCV is performed by holding the microelectrode at a constant potential versus a reference electrode, followed by a rapid increase in potential and an immediate return back to the holding potential. This triangular shaped waveform provides the selectivity intrinsic to FSCV. By locating the potentials at which the maximum cathodic and anodic currents occur, one can distinguish many different neurotransmitters. FSCV coupled with microelectrodes can be used to monitor sub-second changes in neurotransmitter concentration as a result of pharmacological or behavioral manipulations. This unique setup allows for measurements in anesthetized [22] and behaving animals to probe the correlation between behavior and neurotransmission.

Of particular interest is the study of phasic and tonic changes in neurotransmitter concentration. Previously, phasic and tonic changes were measured using electrophysiology [23,24], which is a technique that measures the electrical signals generated during neuronal communication. Unfortunately these signals cannot be accurately correlated with the type of neurotransmitter it employs [25,26], thus requiring an alternate approach to measure the neurotransmitter directly. It has also been suggested that neurons undergoing phasic or burst firing do not have sufficient strength to modify surrounding neuronal circuits [27]; however, it seems likely that phasic firing of dopamine neurons and subsequent release of dopamine has a significant role in learning and memory [28]. Figure 1 (d, f) demonstrates the role that dopamine plays in Pavlovian learning. Increases in dopamine release in the nucleus accumbens shell accompanied cues that predicted natural rewards. For the molecule dopamine, there has been a significant amount of study into understanding the rapid changes that the dopaminergic system undergoes upon repeated stimulation [29]. In these studies, cyclic voltammetry was coupled with microelectrodes to determine the mechanism by which neurons are able to regulate these transient increases and decreases in dopamine release, ultimately determined to be dependent on the dopamine D2 autoreceptor [30].

As a direct result of measuring neurotransmitter release, much can be learned about both natural rewards and drugs of abuse and their effects on neuronal circuitry. Drugs such as cocaine are known to cause increased extracellular concentrations of dopamine over a period of 30 minutes to 1 hour; however, there are additional effects of cocaine that occur on the timescale of a few seconds [31]. Upon administration of cocaine to a behaving rat, both tonic and phasic levels of dopamine increase significantly which can be measured using FSCV [31]. Drugs can also be used to explore the kinetics of neuronal receptor function. The dopamine D2 autoreceptor is responsible for regulating the amount of dopamine released from the neuron. Once dopamine has been released into the synapse, it can bind to the D2 autoreceptor, thereby inhibiting further dopamine release. The kinetics of activation was studied using FSCV by varying the amount of time in between subsequent release events and comparing the amplitude of the first and the second release event [32]. Studying the kinetics of both drugs and receptors can be easily accomplished by directly measuring the concentration of released neurotransmitter [33].

Not all neurotransmitters are released via exocytosis. Adenosine is a molecule produced during metabolism that acts as a chemical messenger. FSCV has been used to measure changes in adenosine concentration as a result of electrical stimulation of the neuron [34]. Other signals that have been detected using FSCV that are not exocytotic include changes in oxygen concentration and changes in physiological pH [7]. Both of these changes have been implicated in blood flow, along with nitric oxide [35,36]. Figure 2 demonstrates how biological changes can be monitored both via optical means as well as through electrochemical measurements. In Figure 2b (middle panel), note how the dilation of the blood vessel correlates with the presence of increased levels of measured nitric oxide Figure 2a (green line). Microelectrodes are now allowing the study of biological phenomena that were previously only accessible through fMRI and other less spatially resolved techniques.

Conclusions and future perspectives

The future of microelectrodes lies in combining the aforementioned detection methods into an electrode array. Valuable information could be obtained by making multiple recordings simultaneously. To some extent this is already occurring; for instance, combined electrophysiology and voltammetry measurements have been performed in behaving animals [37]. Preliminary work using this method has demonstrated the role of dopamine release on intracranial self-stimulation [38]. Additionally, multielectrode arrays for amperometry have been used to measure multiple release sites on cells [39,40]. Potential advancements include combination of iontophoresis and voltammetry to measure changes in catecholamine concentration upon localized pharmacological manipulation.

Electrophysiological arrays have already been developed to measure several neurons involved in motor function simultaneously [41]; however, there are not currently many voltammetric arrays for simultaneously measuring multiple brain regions. The development of a flexible carbon microelectrode should serve to facilitate the development of a sensitive voltammetric array [42]. This would be a logical next step for investigating brain regions that are in close proximity but are structurally distinct such as the nucleus accumbens core and shell. These advancements would allow us to understand better how reward and addiction are governed.

Microelectrodes have played a key role in the advancement of our understanding of the brain. Their unique characteristics have allowed them to be employed in many biological preparations, including many that were not listed in this review [43,44]. The information obtained on the subsecond time scale is unprecedented on the neurobiological time scale. When used with other techniques which can measure tonic changes, microelectrodes provide an excellent means of helping to illustrate a complete picture of neurobiological function.

Acknowledgements

Research in the Wightman laboratory in this area has been supported by NIH.

Bibliography

1. Mosharov EV, Sulzer D. Analysis of exocytotic events recorded by amperometry. Nat Methods 2005;2:651–658. [PubMed: 16118635]
2. Peters JL, Miner LH, Michael AC, Sesack SR. Ultrastructure at carbon fiber microelectrode implantation sites after acute voltammetric measurements in the striatum of anesthetized rats. J Neurosci Methods 2004;137:9–23. [PubMed: 15196823]
3. Wightman RM. Detection technologies. Probing cellular chemistry in biological systems with microelectrodes. Science 2006;311:1570–1574. [PubMed: 16543451]

4. Wu WZ, Huang WH, Wang W, Wang ZL, Cheng JK, Xu T, Zhang RY, Chen Y, Liu J. Monitoring dopamine release from single living vesicles with nanoelectrodes. J Am Chem Soc 2005;127:8914–8915. [PubMed: 15969544]
5. Amatore C, Maisonhaute E. When voltammetry reaches nanoseconds. Anal Chem 2005;77:303A–311A.
6. Shin JH, Weinman SW, Schoenfisch MH. Sol-Gel Derived Amperometric Nitric Oxide Microsensor. Anal Chem 2005;77:3494–3501. [PubMed: 15924380]
7. Venton BJ, Michael DJ, Wightman RM. Correlation of local changes in extracellular oxygen and pH that accompany dopaminergic terminal activity in the rat caudate-putamen. J Neurochem 2003;84:373–381. [PubMed: 12558999]
8. Lee Y, Kim J. Simultaneous electrochemical detection of nitric oxide and carbon monoxide generated from mouse kidney organ tissues. Anal Chem 2007;79:7669–7675. [PubMed: 17877421]
9. Heien ML, Khan AS, Ariansen JL, Cheer JF, Phillips PE, Wassum KM, Wightman RM. Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. Proc Natl Acad Sci U S A 2005;102:10023–10028. [PubMed: 16006505]
10. John CE, Jones SR. Voltammetric characterization of the effect of monoamine uptake inhibitors and releasers on dopamine and serotonin uptake in mouse caudate-putamen and substantia nigra slices. Neuropharmacology 2007;52:1596–1605. [PubMed: 17459426]
11. Westerink RH, Ewing AG. The PC12 cell as model for neurosecretion. Acta Physiol (Oxf) 2008;192:273–285. [PubMed: 18005394]
12. Borges R, Camacho M, Gillis KD. Measuring secretion in chromaffin cells using electrophysiological and electrochemical methods. Acta Physiol (Oxf) 2008;192:173–184. [PubMed: 18021323]
13. Mosharov EV. Analysis of single-vesicle exocytotic events recorded by amperometry. Methods Mol Biol 2008;440:315–327. [PubMed: 18369956]
14. Amatore C, Arbault S, Bonifas I, Guille M, Lemaitre F, Verchier Y. Relationship between amperometric pre-spike feet and secretion granule composition in chromaffin cells: an overview. Biophys Chem 2007;129:181–189. [PubMed: 17587484]
15. Mosharov EV, Gong LW, Khanna B, Sulzer D, Lindau M. Intracellular patch electrochemistry: regulation of cytosolic catecholamines in chromaffin cells. J Neurosci 2003;23:5835–5845. [PubMed: 12843288]
16. Miranda-Ferreira R, de Pascual R, de Diego AM, Caricati-Neto A, Gandia L, Jurkiewicz A, Garcia AG. Single-vesicle catecholamine release has greater quantal content and faster kinetics in chromaffin cells from hypertensive, as compared with normotensive, rats. J Pharmacol Exp Ther 2008;324:685–693. [PubMed: 17962518]
17. Wang CT, Bai J, Chang PY, Chapman ER, Jackson MB. Synaptotagmin-Ca(2+) triggers two sequential steps in regulated exocytosis in rat PC12 cells: fusion pore opening and fusion pore dilation. J Physiol 2006;570:295–307. [PubMed: 16293646]
18. Rizzoli SO, Betz WJ. Synaptic vesicle pools. Nat Rev Neurosci 2005;6:57–69. [PubMed: 15611727]
19. Duncan RR, Greaves J, Wiegand UK, Matskevich I, Bodammer G, Apps DK, Shipston MJ, Chow RH. Functional and spatial segregation of secretory vesicle pools according to vesicle age. Nature 2003;422:176–180. [PubMed: 12634788]
20. Villanueva M, Thornley K, Augustine GJ, Wightman RM. Synapsin II negatively regulates catecholamine release. Brain Cell Biol 2006;35:125–136. [PubMed: 17957479]
21. Moore JM, Papke JB, Cahill AL, Harkins AB. Stable gene silencing of synaptotagmin I in rat PC12 cells inhibits Ca2+-evoked release of catecholamine. Am J Physiol Cell Physiol 2006;291:C270–281. [PubMed: 16467400]
22. Greco PG, Meisel RL, Heidenreich BA, Garris PA. Voltammetric measurement of electrically evoked dopamine levels in the striatum of the anesthetized Syrian hamster. J Neurosci Methods 2006;152:55–64. [PubMed: 16176838]
23. Tobler PN, Fiorillo CD, Schultz W. Adaptive coding of reward value by dopamine neurons. Science 2005;307:1642–1645. [PubMed: 15761155]
24. Schultz W. Behavioral dopamine signals. Trends Neurosci 2007;30:203–210. [PubMed: 17400301]
25. Ungless MA, Magill PJ, Bolam JP. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. Science 2004;303:2040–2042. [PubMed: 15044807]

26. Margolis EB, Lock H, Hjelmstad GO, Fields HL. The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? J Physiol 2006;577:907–924. [PubMed: 16959856]
27. Goto Y, Otani S, Grace AA. The Yin and Yang of dopamine release: a new perspective. Neuropharmacology 2007;53:583–587. [PubMed: 17709119]
28. Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci 2007;10:1020–1028. [PubMed: 17603481]
29. Montague PR, McClure SM, Baldwin PR, Phillips PE, Budygin EA, Stuber GD, Kilpatrick MR, Wightman RM. Dynamic gain control of dopamine delivery in freely moving animals. J Neurosci 2004;24:1754–1759. [PubMed: 14973252]
30. Kita JM, Parker LE, Phillips PE, Garris PA, Wightman RM. Paradoxical modulation of short-term facilitation of dopamine release by dopamine autoreceptors. J Neurochem 2007;102:1115–1124. [PubMed: 17663751]
31. Stuber GD, Wightman RM, Carelli RM. Extinction of cocaine self-administration reveals functionally and temporally distinct dopaminergic signals in the nucleus accumbens. Neuron 2005;46:661–669. [PubMed: 15944133]
32. Phillips PE, Hancock PJ, Stamford JA. Time window of autoreceptor-mediated inhibition of limbic and striatal dopamine release. Synapse 2002;44:15–22. [PubMed: 11842442]
33. Exley R, Cragg SJ. Presynaptic nicotinic receptors: a dynamic and diverse cholinergic filter of striatal dopamine neurotransmission. Br J Pharmacol 2008;153:S283–297. [PubMed: 18037926]
34. Cechova S, Venton BJ. Transient adenosine efflux in the rat caudate-putamen. J Neurochem. 2008
35. Amatore C, Arbault S, Bouret Y, Cauli B, Guille M, Rancillac A, Rossier J. Nitric oxide release during evoked neuronal activity in cerebellum slices: detection with platinized carbon-fiber microelectrodes. Chemphyschem 2006;7:181–187. [PubMed: 16353265]
36. Rancillac A, Rossier J, Guille M, Tong XK, Geoffroy H, Amatore C, Arbault S, Hamel E, Cauli B. Glutamatergic Control of Microvascular Tone by Distinct GABA Neurons in the Cerebellum. J Neurosci 2006;26:6997–7006. [PubMed: 16807329]
37. Cheer JF, Heien ML, Garris PA, Carelli RM, Wightman RM. Simultaneous dopamine and single-unit recordings reveal accumbens GABAergic responses: implications for intracranial self-stimulation. Proc Natl Acad Sci U S A 2005;102:19150–19155. [PubMed: 16380429]
38. Cheer JF, Aragona BJ, Heien ML, Seipel AT, Carelli RM, Wightman RM. Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. Neuron 2007;54:237–244. [PubMed: 17442245]
39. Hafez I, Kisler K, Berberian K, Dernick G, Valero V, Yong MG, Craighead HG, Lindau M. Electrochemical imaging of fusion pore openings by electrochemical detector arrays. Proc Natl Acad Sci U S A 2005;102:13879–13884. [PubMed: 16172395]
40. Zhang B, Adams KL, Luber SJ, Eves DJ, Heien ML, Ewing AG. Spatially and temporally resolved single-cell exocytosis utilizing individually addressable carbon microelectrode arrays. Anal Chem 2008;80:1394–1400. [PubMed: 18232712]
41. Lebedev MA, Nicolelis MA. Brain-machine interfaces: past, present and future. Trends Neurosci 2006;29:536–546. [PubMed: 16859758]
42. Hermans A, Wightman RM. Conical tungsten tips as substrates for the preparation of ultramicroelectrodes. Langmuir 2006;22:10348–10353. [PubMed: 17129002]
43. de Diego AM, Gandia L, Garcia AG. A physiological view of the central and peripheral mechanisms that regulate the release of catecholamines at the adrenal medulla. Acta Physiol (Oxf) 2008;192:287–301. [PubMed: 18005392]
44. Schmitz Y, Benoit-Marand M, Gonon F, Sulzer D. Presynaptic regulation of dopaminergic neurotransmission. J Neurochem 2003;87:273–289. [PubMed: 14511105]

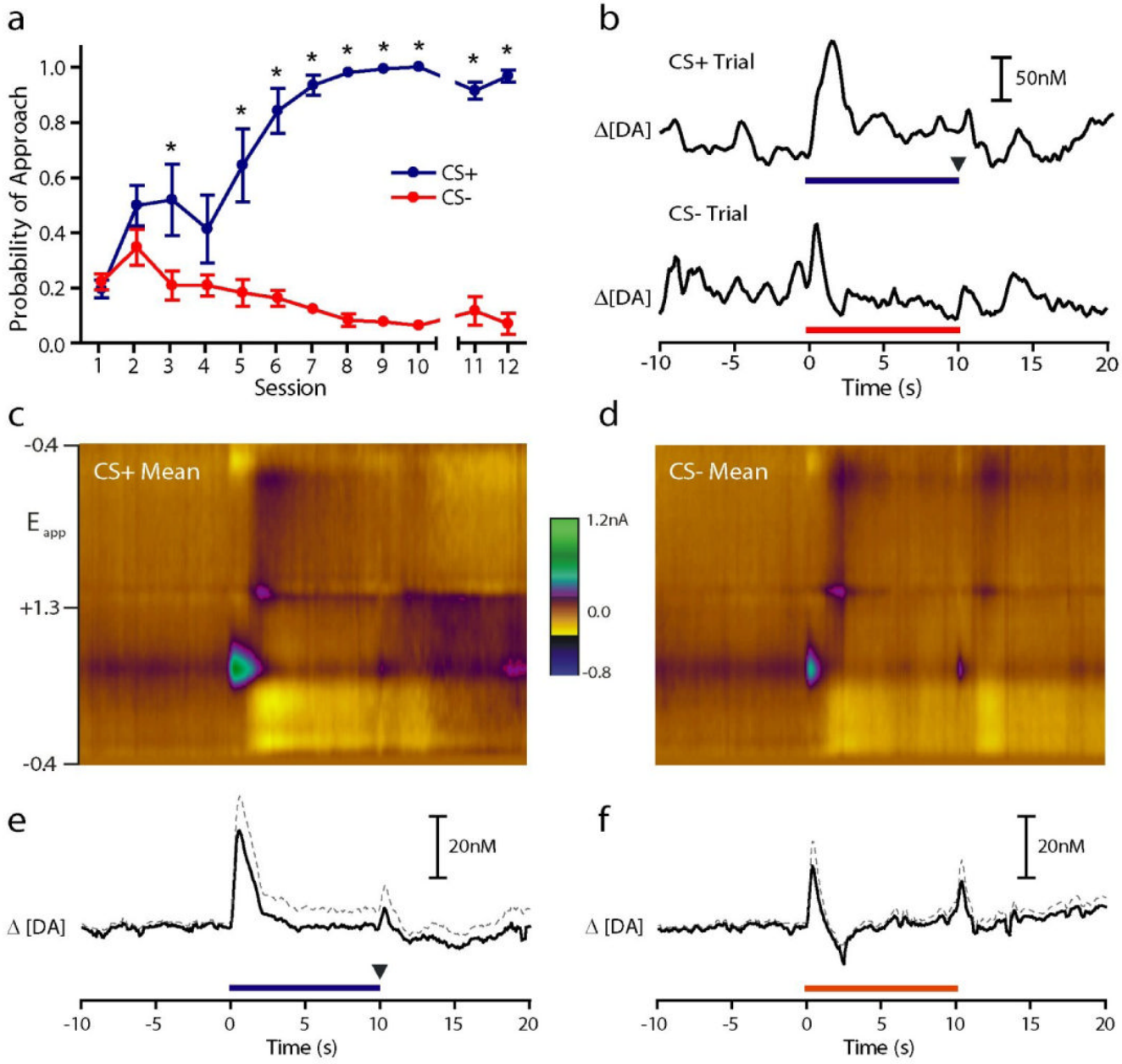

Figure 1.

a) Behavioral discrimination (mean (SEM) of approach probability) between conditioned stimuli based on predictive value. Rats approached the predictive CS+ significantly more than the nonpredictive CS- in sessions 6–12. After ten conditioning sessions, animals underwent surgery for implantation of the voltammetric recording apparatus (indicated by break in graph). (**b**) Representative changes in dopamine signaling during individual CS+ (top) and CS- (bottom) trials. (**c**) Three-dimensional representation of mean electrochemical data collected during reward-predictive CS+ trials. CS+ presentations evoked an immediate rise in signal that returned to baseline levels in seconds. (**d**) Mean (SEM) increase in [DA] evoked by CS+ onset was significantly greater than baseline [DA] at 0.3–1.4 s after CS+ onset. No increase in signal was observed relative to reward delivery. (**e**) Three-dimensional representation of mean electrochemical data collected during CS- trials. CS- presentations evoked relatively smaller

increases in signal. (**f**) Mean (SEM) [DA] also changed after CS- onset. Reprinted with permission from (28)

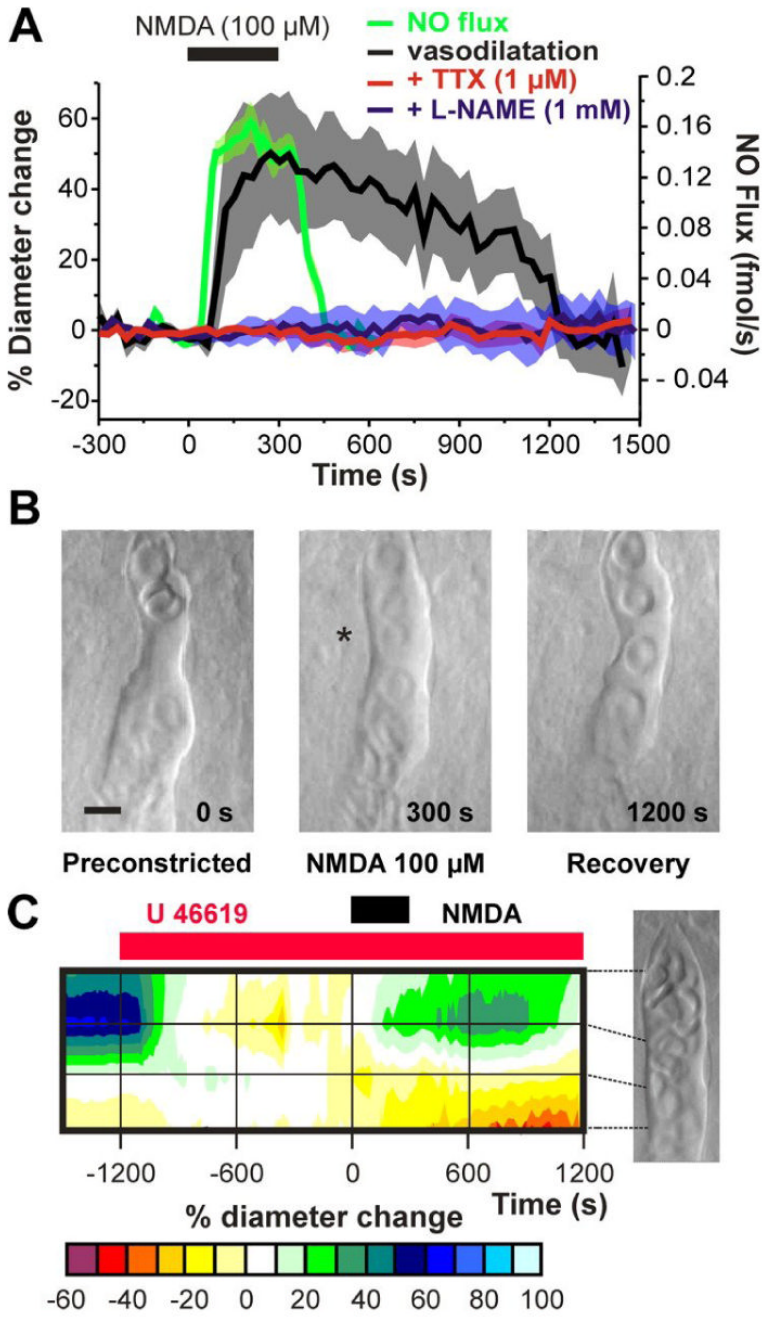

Figure 2.

NO mediates the NMDA-induced vasodilations. **(a)** Left axis, The mean vascular dilation induced by NMDA (n = 7; black) is abolished by TTX (1 μM; n = 7; red) or L-NAME (1 mM; n = 4; blue). Right axis, NO flux (green trace) elicited by NMDA (n = 5). The SEM envelopes the mean traces. **(b)** Infrared images of an intraparenchymal cerebellar blood vessel preconstricted with U46619 (75 nM) that reversibly dilated to NMDA (100 μM) application. Scale bar, 10 μm. The asterisk indicates a region of high vascular reactivity. **(c)** Spatiotemporal response of the blood vessel shown in **(b)**. Note the spatially restricted constriction (from blue to yellow) under U46619 (75 nM) application (red box) that reversed to a dilation (from yellow to green) after NMDA application (black box). Right, Infrared image of the blood vessel before

U46619 application with the locations of measurements indicated by black lines. Reprinted with permission from (35).